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Effect of Sodium Polyphosphates on Growth of *Listeria monocytogenes*

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ABSTRACT

Evaluation of sodium polyphosphates (SPP), multifunctional food additives, indicated that only the higher polymers sodaphos, hexaphos, and glass H (average chain length = 6, 13, and 21, respectively) significantly inhibited growth of *Listeria monocytogenes*. The effect of the three compounds (0-2%) on growth of *L. monocytogenes* was studied in brain heart infusion + 0.3% glucose medium, pH 6.0, at 28, 19, 10, and 5°C. The organism grew well under all test conditions in the absence of SPP. Hexaphos and glass H were considerably more inhibitory than sodaphos. The most pronounced effect of SPP was on lag times, which increased with increasing SPP concentration and decreasing temperature. At 10°C, addition of 0.3% hexaphos or glass H increased lag time from 22 h to 197 and 186 h, respectively, and no growth was observed after 40 d in the presence of 2.0% of these compounds. Addition of 2.0% NaCl increased the inhibitory effect of SPP but had little effect on growth in SPP-free media. Results suggest that high molecular weight SPP may be useful in controlling the growth of *L. monocytogenes*, particularly at low temperatures and in combination with NaCl.

Polyphosphates, which are polymers of phosphoric acid, are widely used food additives that have the distinction of being able to act as buffers, emulsifiers, dispersants, antioxidants, and sequestrants. These compounds, particularly sodium pyrophosphate and sodium tripolyphosphate, are used extensively in the meat industry as moisture binding agents. U.S. Department of Agriculture regulations currently limit polyphosphates in meat and poultry products to 0.5% based on functional properties of these compounds (*16*). The properties of polyphosphates and their uses in food have been reviewed by Ellinger (*3*) and Molins (*12*). Polyphosphates also possess antimicrobial activity. There are indications that polyphosphates may be useful as antimicrobial agents in foods, although at present these compounds are not classified or approved as preservatives or antimicrobial agents (*12*). Available literature on the antimicrobial activity of food phosphates has been reviewed by Ellinger (*3*), Molins (*12*), Hargreaves et al. (*7*), and Tompkin (*15*). Gram-positive bacteria are inhibited by polyphosphates, whereas little or no inhibition of gram-negative bacteria has been observed (*2,13*). Particular attention has been devoted to *Clostridia* (*17*) and *Staphylococcus aureus* (*8*), and results indicate that polyphosphates may inhibit bacterial growth and toxin formation. Inhibition of fungi by polyphosphates has also been reported (*9,11*).

Recent outbreaks of foodborne listeriosis, some resulting in fatalities, have focused attention on the need to control *Listeria monocytogenes* in foods. The purpose of this investigation is to determine whether or not polyphosphates are capable of inhibiting this gram-positive foodborne pathogenic bacterium. The experiments were carried out in a bacteriological medium, and the influence of temperature and added sodium chloride was assessed.

MATERIALS AND METHODS

Microorganism

Listeria monocytogenes Scott A was used throughout the study. To prepare the inoculum, the organism was cultured for 18-24 h at 37°C in brain heart infusion (Difco) medium supplemented with 0.3% glucose, and the culture was diluted with sterile 0.1% peptone water.

Test chemicals

Sodium tripolyphosphate and the sodium polyphosphates sodaphos, hexaphos, and glass H were obtained from FMC Corp., Philadelphia, PA. Sodium pyrophosphate decahydrate and sodium hypophosphite hydrate were from Mallinckrodt Co., Paris, KY. Aqueous solutions of test compounds were prepared, sterilized by filtration through a 0.2-micron cellulose acetate membrane filter (Nalge Co., Rochester, NY), and added to sterile media to obtain the desired concentration. When necessary, solutions of the test compounds were adjusted to pH 6.0 before addition to media. Magnesium sulfate heptahydrate was from J. T. Baker Co.

Culture techniques

Brain heart infusion (BHI), 37 g, dissolved in ca. 800 ml water, was supplemented with 3 g glucose and 0 or 20 g sodium chloride. The base quantities of glucose and sodium chloride in BHI are 2 and 5 g, respectively. The solution was adjusted to pH 6.0 with 2 N HCl and diluted with water to 900 ml. The medium was then dispensed in 90-ml portions into 500-ml Erlenmeyer flasks. The flasks, capped with foam plugs, were sterilized by

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autoclaving for 15 min at 121°C. Sterile water or 10% sodium polyphosphate solutions (10 ml total volume) were added to the sterile media. The final media contained 0.3% glucose; 0.5 or 2.5% sodium chloride; and 0, 0.3, 0.5, or 1.0% sodium polyphosphate compound. Media containing a final concentration of 2.0% sodium polyphosphate were prepared by addition of 20 ml of 10% polyphosphate solution to 80 ml of sterile medium prepared as described above but diluted to 800 ml. All flasks were inoculated with 1 ml of a diluted 18-24 h culture of *L. monocytogenes* to an initial level of 10^3 CFU/ml. All flasks were then incubated on a rotary shaker (150 rpm) at 5, 10, 19, or 28°C.

At appropriate intervals samples were withdrawn from each flask by means of a pipet, and the microbial populations were determined by surface plating on tryptose agar (Difco) using a Spiral Plater (Spiral System Instruments, Inc., Bethesda, MD). The plates were incubated for 24 h at 37°C and counted.

For experiments involving added Mg^{2+}, appropriate volumes of filter-sterilized aqueous solutions of $MgSO_4.7H_2O$, glass H, or sterile water (total volume, 5 ml) were added to 45 ml of sterile media prepared to 90% of final dilution as described above.

Curve fitting and determination of growth parameters

Bacterial growth curves were generated from the experimental data using the Gompertz equation (*6*) in conjunction with ABACUS, a nonlinear regression program that employs a Gauss-Newton iteration procedure. This FORTRAN-based program was developed by W. C. Damert (U.S. Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA), and copies are available upon request. The Gompertz parameter values (A, B, C, M) were subsequently used to calculate exponential growth rates [($\log_{10}$ CFU/ml)/h], generation times (h), lag times (h), and maximum population densities ($\log_{10}$ CFU/ml) as described by Gibson et al. (*6*).

RESULTS AND DISCUSSION

The structure and properties of the sodium polyphosphates studied are shown in Table 1. Initial experiments indicated that these compounds inhibited the growth of *L. monocytogenes* in BHI media. Inhibition increased with decreasing temperature and was greater at pH 6 than at pH 7. Growth of *L. monocytogenes* in the presence of 0.5% of the test compounds in media of pH 6.0 at 28°C is compared in Table 2. While sodium pyrophosphate (n = 2) and sodium tripolyphosphate (n = 3) had minimal activity, the higher polymers sodaphos, hexaphos, and glass H significantly inhibited the growth of *L. monocytogenes*. Previously, Kohl and Ellinger (*10*) reported that medium chain length polyphosphates (n = 16 to 37) were particularly efficient microbial inhibitors. Sodium hypophosphite, reported to possess antimicrobial properties (*14*), was also tested but showed no activity against *L. monocytogenes* (Table 2).

TABLE 1. *Structure and properties of sodium polyphosphates.*

$Na_{(n+2)}\ P_{(n)}\ O_{(3n+1)}$

	Chain length	P_2O_5 (%)	Sol. in H_2O, g/Kg H_2O (25°C)	pH, 1% Soln
Pyrophosphate	n = 2	53.1	80	10.3
Tripolyphosphate	n = 3	57.3	150	9.9
Sodaphos	n = 6	64.1	Infinite	7.8
Hexaphos	n = 13	67.5	Infinite	7.0
Glass H	n = 21	69.0	Infinite	6.5

TABLE 2. *Effect of 0.5% sodium polyphosphates and sodium hypophosphite on growth of* L. monocytogenes *at 28°C in BHI + 0.3% glucose, pH 6.0, 0.5% NaCl.*

	Bacterial population[a], $\log_{10}$ CFU/ml				
	Incubation time, (h)				
	24	48	116	166	360
Control	8.82	9.15	-	-	-
Sodium pyrophosphate[b]	7.68	8.90	9.00	-	-
Sodium tripolyphosphate	5.79	8.86	8.40	-	-
Sodaphos	2.90	4.62	8.61	-	-
Hexaphos	2.97	4.25	8.46	-	-
Glass H	2.74	2.56	3.26	4.01	8.32
Sodium hypophosphite	9.12	9.21	-	-	-

[a] Initial bacterial count, $\log_{10}$ CFU/ml = 3.25.
[b] $Na_4P_2O_7.10H_2O$, 0.84%, was used = 0.5% $Na_4P_2O_7$.

Since sodaphos, hexaphos, and glass H showed significant inhibitory effect, they were tested in more detail at 28, 19, 10, and 5°C at pH 6.0. The effect of added NaCl on growth at 28 and 19°C was also determined. An example of the experimental growth data and the corresponding fitted growth curves for cultures at 19°C containing 0, 0.3, 0.5, and 1.0% glass H in combination with 2.5% NaCl is shown in Fig. 1. A comparison of the effect on *L. monocytogenes* of 0, 0.3, 0.5, and 1.0% sodaphos, hexaphos, and glass H, with or without added NaCl, is presented in Fig. 2a,b for growth at 28°C and in Fig. 3a,b for growth at 19°C. Bacterial growth occurred in all cases in the presence of the polyphosphates; however, greater inhibition was obtained with hexaphos and glass H than with sodaphos at both temperatures. Addition of the polyphosphates resulted in decreased exponential growth rates, increased generation times, and increased lag times. The effect of increasing concentration of polyphosphate was most evident with lag times (Fig. 2a and 3a).

Addition of NaCl had a relatively minor effect on the growth parameters of *L. monocytogenes* in the absence of polyphosphates (Fig. 2 and 3). Previously Buchanan et al. (*1*) observed that *L. monocytogenes* grows readily at NaCl con-

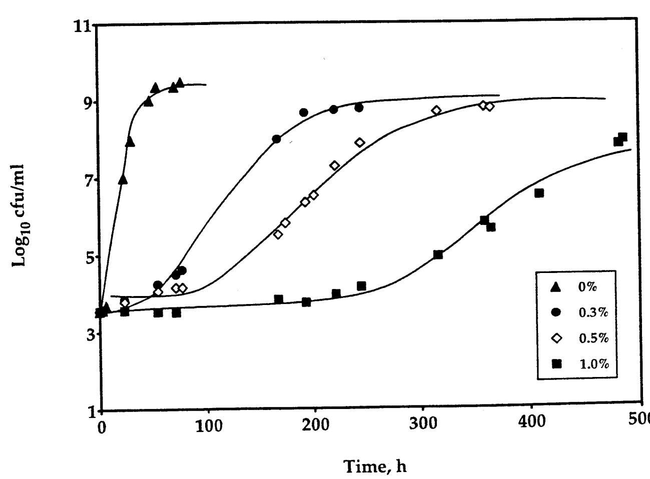

Figure 1. *Effect of Glass H concentration on growth of* L. monocytogenes *at 19°C in BHI + 0.3% glucose (pH 6.0) with 2.5% NaCl.*

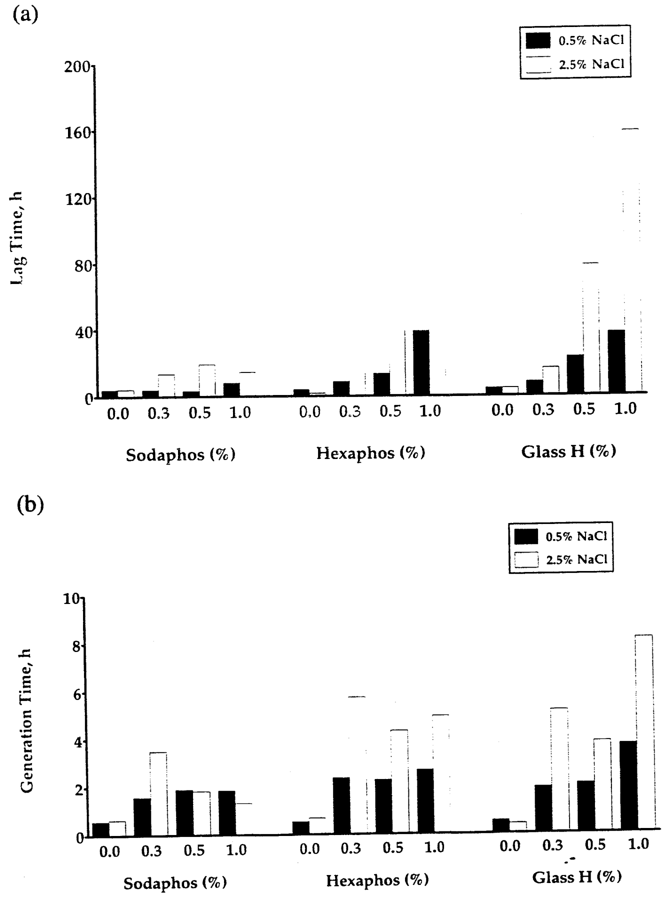

Figure 2. *Effect of sodium polyphosphates (0, 0.3, 0.5, 1.0%) and sodium chloride (0.5, 2.5%) on growth kinetics parameters of* L. monocytogenes *in BHI + 0.3% glucose, pH 6.0, at 28°C. a = lag time; b = generation time.*

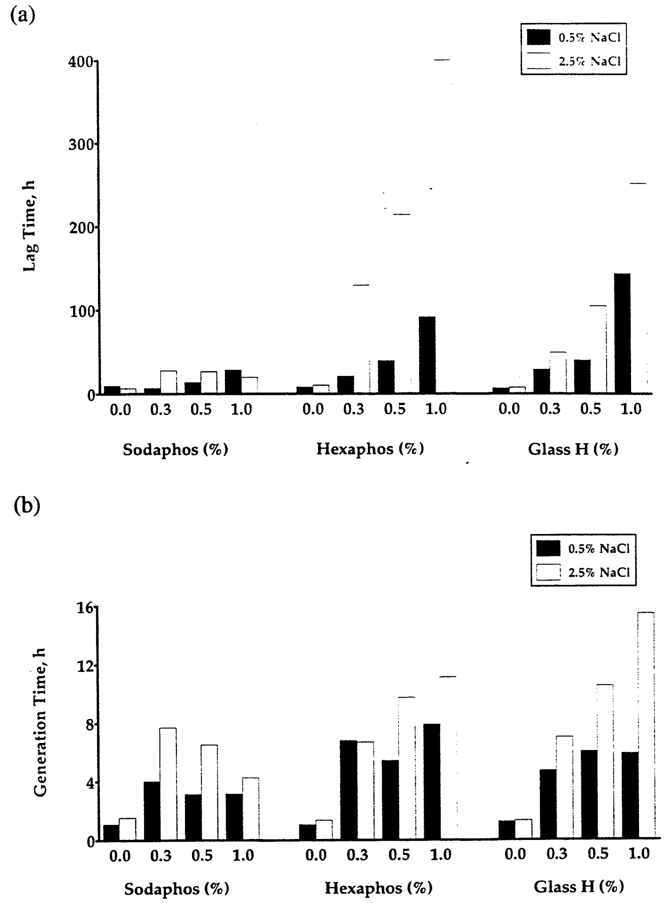

Figure 3. *Effect of sodium polyphosphates (0, 0.3, 0.5, 1.0%) and sodium chloride (0.5, 2.5%) on growth kinetics parameters of* L. monocytogenes *in BHI + 0.3% glucose, pH 6.0, at 19°C. a = lag time; b = generation time.*

Figure 3b.

centrations as high as 4.5% and that NaCl did not have a major effect on the growth kinetics of this organism. However, addition of NaCl greatly enhanced the inhibitory effect of polyphosphates, producing a dramatic increase in lag times, especially in cultures containing hexaphos and glass H. Firstenberg-Eden et al. (*5*) reported that combinations of NaCl and sodium pyrophosphate were synergistic in inhibiting *Moraxella-Acinetobacter*, whereas combinations of NaCl and sodium tripolyphosphate produced an additive effect.

Polyphosphates did not produce any major effect on the maximum population densities. The maximum population density of control cultures at both NaCl concentrations and temperatures had an average value of $\log_{10}$ CFU/ml = 9.6 (range, 9.4-9.9), while the corresponding cultures containing sodaphos, hexaphos, and glass H had an average value of $\log_{10}$ CFU/ml = 9.1 (range, 8.6-9.5).

The three sodium polyphosphates tested were effective inhibitors of *L. monocytogenes* at low temperatures (Table 3). The organism grew rapidly at 10°C in control media; however, no growth was obtained after 40 d in the presence of 2.0% hexaphos or 1.0 and 2.0% glass H, and levels of 0.5% of the compounds effectively delayed growth. The organism also grew readily at 5°C in the absence of polyphosphates, while addition of 0.3% of sodaphos, hexaphos, or glass H considerably delayed growth. No growth was obtained after 66 d at 5°C in cultures containing 1% hexaphos or glass H and only slow growth in the presence of 1% sodaphos. In all

TABLE 3. *Effect of sodium polyphosphates on growth of* L. monocytogenes[a] *in BHI + 0.3% glucose, pH 6.0, 0.5% NaCl, at low temperatures.*

	Polyphosphate (%)	Time to reach $\log_{10}$ CFU/ml = 8 (days)
10°C		
Control	-	4
Sodaphos	0.5	16
	1.0	15
Hexaphos	0.5	35
	1.0	45
	2.0	No growth in 40 d
Glass H	0.5	42
	1.0	No growth in 40 d
	2.0	No growth in 40 d
5°C		
Control	-	8.5
Sodaphos	0.3	28
	1.0	66 (log = 6)
Hexaphos	0.3	45
	1.0	No growth in 66 d
	0.3	45
Glass H	1.0	No growth in 66 d

[a] Initial level, $\log_{10}$ CFU/ml, was 3.4 at 10°C and 4.2 at 5°C.

cases where no increase in population occurred, the organism remained viable.

Since polyphosphates are effective chelating agents, they may exert their inhibitory effect by removing essential metals from cation-binding sites on the cell walls of microorganisms (*9*). Reversal of growth inhibition was often obtained by the addition of metal cations, particularly Mg^{2+}, to media containing inhibitory concentrations of polyphosphates (*4,8,9,13*). Gram-positive bacteria have a much greater requirement for Mg^{2+} than do gram-negative bacteria (*18*). This may be a contributing factor to the greater sensitivity to polyphosphates of gram-positive bacteria compared to gram-negative bacteria (*9*). Addition of Mg^{2+} to *L. monocytogenes* cultures in BHI + 0.3% glucose, pH 6.0, 0.5% NaCl, containing 0.5% sodium polyphosphate glass H, resulted in significant reversal of growth inhibition even at a level of 0.001 M Mg^{2+}, and rapid growth was obtained at higher concentrations (Table 4). Growth of *L. monocytogenes* was similar in media containing 0-0.1 M added $MgSO_4$ in the absence of polyphosphates (data not shown). Jen and Shelef (*8*) reported that BHI broth contains 0.35 mM Mg. We did not investigate the effect of other cations. However, Knabel et al. (*9*) reported that growth of *L. monocytogenes* Scott A was inhibited on BHI agar containing 1% tetrasodium pyrophosphate, but growth occurred upon addition of Fe^{3+}.

TABLE 4. *Effect of added magnesium ion on growth inhibition of* L. monocytogenes *by sodium polyphosphate Glass H in BHI + 0.3% glucose, pH 6.0, 0.5% NaCl, at 19°C.*

	Bacterial population[a], $\log_{10}$ CFU/ml		
	Incubation time (h)		
	48	145	307
0.5% Glass H	3.45	4.94	8.39
0.5% Glass H + 0.001 M $MgSO_4$	4.68	8.77	-
0.5% Glass H + 0.002 M $MgSO_4$	5.21	9.22	-
0.5% Glass H + 0.005 M $MgSO_4$	> 6[b]	9.26	-
Control	9.44	-	-

[a] Initial bacterial count, $\log_{10}$ CFU/ml = 3.58.
[b] The number of colonies on plates using undiluted culture were too numerous to obtain an accurate count.

Although the activity against *L. monocytogenes* of the longer chain sodium polyphosphates examined (n = 6, 13, 21) appears to be bacteriostatic rather than bactericidal at the concentrations tested (up to 2.0%), these compounds may be useful in inhibiting the proliferation of this pathogen in food, particularly if used in combination with NaCl, other antimicrobial agents, and low temperatures.

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